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Detection of Human Herpesvirus 8 DNA Sequences in Peripheral Blood Mononuclear Cells of Children

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Human herpesvirus 8 (HHV-8) DNA sequences were examined in peripheral blood mononuclear cell (PBMC) DNA samples of 56 children, 15 healthy adults, and 10 renal transplant patients by the polymerase chain reaction (PCR). The PCR amplification was carried out using the published $KS330_{233}$ primer pairs to amplify HHV-8 DNA sequences. The PCR-amplified products were confirmed by Southern blot hybridization with radiolabeled 233 bp HHV-8 DNA fragment, which was cloned and sequenced from the PCR-amplified product of Kaposi's sarcoma tissue. Six PCR-amplified product of four children and two renal transplant patients were cloned and sequenced. HHV-8 DNA sequences were detected in 36 of 56 (64%) normal children, in 12 of 15 (80%) healthy adults, and in all 10 renal transplant patients by Southern blot hybridization of PCR-amplified products. Six PCR-amplified products were confirmed by sequencing. These results suggest that HHV-8 infection is prevalent in the Japanese population with infection occurring in early childhood. ***J. Med. Virol. 53:81–84, 1997.*** © Wiley-Liss, Inc.

KEY WORDS: human herpesvirus 8 (HHV-8); Kaposi's sarcoma-associated herpesvirus (KSHV); polymerase chain reaction (PCR)

INTRODUCTION

Recently, Chang et al. [1994] identified putative herpesvirus-like DNA sequences in Kaposi's sarcoma (KS) tissues by the method of representational difference analysis. The DNA sequences, designated Kaposi's sarcoma-associated herpesvirus (KSHV)/human herpesvirus 8 (HHV-8) DNA sequences, are partially homologous to *Herpesvirus saimiri* and Epstein-Barr virus (EBV). The DNA sequences were detected in 90% of KS tissues and 15% of non-KS tissues in HIV-infected individuals [Chang et al., 1994]. Thereafter, the DNA sequences were detected not only in AIDS-associated KS but also in classic and endemic KS [Ambroziak et al., 1995; Su et al., 1995; Huang et al., 1995; Moore and Chang, 1995], suggesting that HHV-8 is causally associated with KS. HHV-8 DNA sequences were also detected in the body cavity–based lymphoma of HIV-infected patients [Cesarman et al., 1995] and in samples of multicentric Castleman's disease [Soulier et al., 1995]. Moreover, the sequences were detected in various skin lesions of transplant patients [Rady et al., 1995] and in the semen of HIV-negative healthy men [Lin et al., 1995], suggesting that HHV-8 is a widespread latent virus. However, controversy exists currently on whether HHV-8 is ubiquitous in the healthy population, or infection with this virus is restricted to certain diseases. The purpose of this study was to determine whether HHV-8 DNA sequences are present in perpheral blood mononuclear cells (PBMCs) of young Japanese children.

PATIENTS AND METHODS

DNA Samples

PBMC DNA samples of 56 children with acute febrile illnesses, aged 2 months to 6 years, 15 healthy adults, aged 25 to 40 years, and 10 renal transplant patients, aged 23 to 41 years were examined. Acute febrile illnesses include mainly bacterial respiratory infections which are not associated with HHV-8 infection. None of the children, healthy adults, or renal transplant patients were associated with HIV and KS. The renal transplant patients were treated with basic immunosuppressive therapy after transplantation. PBMCs were obtained by centrifugation of heparinized peripheral blood on a Ficoll-Conray gradient. The KS sample (KS-M) of a Japanese classic KS patient [Koizumi et al., 1996] was used for the positive control. Human DNA samples of EBV genome-positive Raji cells, EBV genome-negative Ramos cells and Molt-4 cells, and human embryonic fibroblast cells were used for the negative controls. The genomic DNA was extracted from these cells and tissue blocks by digestion with proteinase K, extraction with phenol-chloroform, and precipitation with ethanol.

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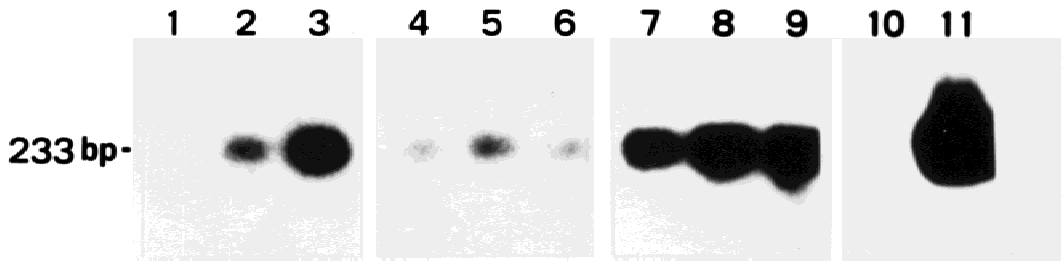

Fig. 1. Southern blot hybridization of the PCR-amplified products. **Lanes 1–3,** PBMC DNA of children. **Lanes 4–6,** PBMC DNA of healthy adults. **Lanes 7–9,** PBMC DNA of renal transplant patients. **Lane 10,** Raji cell DNA, negative. **Lane 11,** Kaposi's sarcoma DNA, positive control.

Polymerase Chain Reaction (PCR)

PCR primers (the primer set for $KS330_{233}$; bp 987 to 1,006 and bp 1,200 to 1,219) were chosen to amplify a 233 bp fragment of the KS330 Bam sequence [Chang et al., 1994]. The PCR reaction mixture consisted of 200 μmol of each deoxyribonucleotide, 2.5 U of *Taq* polymerase, 50 nmol/L of potassium chloride, 10 mmol/L of Tris-HCl (pH 8.3), 1.5 mmol/L of magnesium chloride, 0.01% (wt/vol) of gelatin, 20 pmol of each primer, and approximately 1 μg of DNA in a volume of 100 μl. The PCR conditions were as follows: 94°C for 3 min followed by 35 cycles of 94°C for 1 min, 54°C for 1 min and 72°C for 1 min. The reactions were terminated by a 7-min extension at 72°C. Aliquots (12 μl) of the PCR-amplified product were subjected to electrophoresis through a 1.8% agarose gel and run at 50 V for 4 hr. The gel was stained with ethidium bromide for 5 min, and the DNA bands were visualized under ultraviolet light.

Southern Blot Hybridization

The PCR-amplified products were also analyzed by Southern blot hybridization to confirm the specificity and improve the sensitivity. The cloned and sequenced fragment from the PCR-amplified product of KS-M was used for the probe of Southern blot hybridization. The cloned fragment was labeled with ^{32}P-dCTP using a Nick translation kit (Takara). The PCR-amplified product was transferred onto a nitrocellulose membrane filter. The filter was hybridized with the ^{32}P-labeled cloned fragment of HHV-8 DNA for 24 hr at 42°C in 1 × standard saline citrate (SCC) (0.15 M sodium chloride and 0.015 M sodium citrate), 50% formamide, 0.5% sodium dodecyl sulphate (SDS), and heat-denatured salmon sperm DNA (100 μg/ml). After hybridization, the filter was washed three times at room temperature in 0.1 × SSC with 0.1% SDS, then incubated three times for 1 hr at 56°C. The filter was then dried and exposed to X-ray film at −80°C.

Cloning and Sequencing

The PCR-amplified products from KS-M and PBMCs of four children and two renal transplant patients were cloned using the pCR™II TA cloning kit (Invitrogen). The cloned fragment was sequenced by using an ABI PRISM™ Dye terminator cycle sequencing ready reaction kit (Perkin Elmer) with an ABI 373A automated sequencer (Applied Biosystems). Both sense and antisense strands of the cloned fragments were sequenced.

RESULTS

Direct Gel Analysis of PCR-Amplified Products

The HHV-8 DNA sequences, as indicated by the 233 bp DNA band identical to that of the positive control, were detected in eight of 56 (14%) children on ethidium bromide–stained gels. While positive signals were visible in eight of 10 (80%) renal transplant patients, no signal was visible in the 15 healthy adults. All of the positive signals detected in the eight children and eight renal transplant patients were relatively weak compared with the strongly positive signal in KS-M.

Southern Blot Hybridization of PCR-Amplified Products

The detection of a strong band in the positive control indicated a high copy-number of the HHV-8 DNA sequences in KS-M (Fig. 1). All the negative control cells were negative for these sequences despite prolonged exposure of the autoradiographed membrane. Southern blot hybridization revealed the presence of HHV-8 DNA sequences in 36 of 56 (64%) children. The intensities of the hybridization signals varied among PBMC DNA samples of children. The prevalence of HHV-8 DNA sequences in 26 children younger than two years and 30 children older than two years was 38% and 87%, respectively (Table I). The DNA samples of 12 of 15 healthy adults and all renal transplant patients were positive for HHV-8 DNA sequences. Faint positive signals were recognized in DNA samples of the health adults by long exposure of the autoradiographed membrane. DNA samples of the renal transplant patients gave much stronger signals of the hybridization than those from the healthy adults. These results suggest that the quantity of HHV-8 DNA sequences was consistently higher in PBMCs of the renal transplant patients than those of the healthy adults. HHV-8 DNA sequences were detected reproducibly by the PCR and subsequent Southern blot analysis in a repeat study. Raji cells for negative control were always examined in parallel and were never positive, indicating that the results of Southern blot analysis were not due to contamination.

TABLE I. Prevalence of HHV-8 DNA Sequences in Peripheral Blood Mononuclear Cells by Southern Blot Hybridization of PCR-Amplified Products

Group	Detected/total (%)
Children	
<2 years of age	10/26 (38)
>2 years of age	26/30 (87)
Total	36/56 (64)
Healthy adults	12/15 (80)
Renal transplant patients	10/10 (100)

Sequencing

All cloned fragments had a point mutation at nucleotide position 1,033 (C to T) compared with the prototype sequences [Chang et al., 1994]. The base change at this position results in a proline to leucine substitution. In addition, the cloned fragment from one child had another point mutation at nucleotide position 1,132 (A to G). The base change at this position results in an aspartic acid to glycine substitution. The cloned fragment from KS-M also had a point mutation at nucleotide position 1,019 (C to T). The base change at this position does not involve an amino acid substitution [Koizumi et al., 1996].

DISCUSSION

Seven herpesviruses have been isolated from humans. The human herpesviruses except for herpes simplex virus type 2 (HSV-2) are ubiquitous and infect individuals of all ages, in all parts of the world regardless of socioeconomic or cultural background. The fundamental property of all herpesviruses is their ability to become latent and persist in an apparently inactive state for up to an entire lifetime, then to reactivate when stimulated under certain immunosuppressive conditions.

Whitby et al. [1995] reported HHV-8 was present in PBMCs of 52% of KS patients and in none of 134 healthy donors by nested PCR. However, Bigoni et al. [1996] reported that HHV-8 was present in PBMCs of 9% of healthy donors by both PCR and nested PCR. Decker et al. [1996] recently showed the presence of HHV-8 in PBMCs of four of five allograft patients and three of five health donors by PCR, suggesting that HHV-8 is quite common in the general population. The true prevalence of this newly discovered virus is still a debated issue. In this study, the high prevalence of HHV-8 in PBMCs provides evidence that HHV-8 is a ubiquitous and widespread virus at least in Japan, like the other herpesviruses. Furthermore, most HHV-8 DNA sequences in PBMCs were detectable by Southern blot hybridization but not by direct gel analysis of the PCR-amplified products, suggesting low copy-numbers of the HHV-8 DNA sequences in the PBMC DNA samples. The disparity in the detection rate between our result and those of the other laboratories might reflect our technical ability to detect the genome at low levels. Southern blot analysis using cloned fragments radio-labeled by nick translation appears to be more sensitive than that using end-labeled internal oligonucleotide. Another factor in the discrepancy may be geographic differences. Recent serological studies showed that HHV-8 prevalence rates in the general population were very low in Britain and North America and high in Africa [Simpson et al., 1996; Lennette et al., 1996]. Primary EBV infections occur in approximately 70% of children by the age of two years in Japan. The age of prevalence for primary infection with EBV, which is partially homologous to HHV-8, is distinctly different between Japan and western countries.

The high frequency of HHV-8 DNA sequences in our series of children with acute febrile illnesses is due, at least in part, to the different geographic distribution of the putative HHV-8. HHV-8 may infect latently the Japanese population and may be reactived by immunosuppression or other stimuli. Lennette et al. [1996] suspected two modes of transmission of HHV-8, a sexual and a non-sexual route. The high prevalence of HHV-8 infection suggested that HHV-8 is frequently transmitted through a non-sexual route in early childhood in Japan. Our study does not exclude, however, the possibility that HHV-8 play a causal role in the pathogenesis of different forms of KS among widely varying populations, because EBV, which is a ubiquitous virus, is causally associated with Burkitt's lymphoma and nasopharyngeal lymphoma.

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